FINAL TECHNICAL REPORT
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FRANK W. BOOTH, PH.D.

P-8

1977 Confirmed that the length of the muscle in the immobilized limb determines the amount of atrophy

Confirmed that muscles atrophy in shortened positions during immobilization; show that stretching results in transient hypertrophy in "fast-twitch" muscle; confirm that stretching results in maintained hypertrophy in "slow-twitch" muscle

Showed that the time for 50% of the atrophy that will eventually occur, occurs by the end of the first week of limb immobilization

Showed that the t 1/2 of the loss of cytochrome c content and of citrate synthase content in muscles of immobilized limbs is about 4 days. These proteins are lost more rapidly than are the average loss of other proteins

Showed that myoglobin concentration increases in "fast-twitch" muscle of immobilized limbs

1978–1979 Publications on the recovery of muscle from limb immobilization

1979– Showed that the fractional rate of protein synthesis in skeletal muscle of immobilized limbs decreased about 40% during the first 6 h limb immobilization

Showed that total RNA did not change during the first 6 hours of immobilization

1980– Showed that State III respiration of subsarcolemmal mitochondria from skeletal muscle decreased after 2 days, but not after 1 day of hindlimb immobilization

Confirmed that the respiratory control index decreases in muscle of immobilized limbs

Showed some limitations of using *in vitro* muscles for the study of protein synthesis and degradation, that is

- Cutting muscle fibers decreases rates of protein synthesis
- Cutting muscle fibers increases protein degradation rates (56)
- Stretching 35–45 mg muscles during *in vitro* incubation prevents much of the decrease in ATP and CP concentrations that occur in unstretched muscle of this size *in vitro*

1981– Showed that the fractional rate of protein synthesis in skeletal muscles of immobilized limbs remains decreased at about the same value (decrease was about 40%) from the 6th hour to the 7th day of hindlimb immobilization

Showed that muscle protein synthesis returned to control levels during the first 6 hours after ending a 7-day period of hindlimb immobilization

Showed that exercise of sufficient duration resulted in a further increase in protein synthesis in muscles recovering from a previous period of immobilization

1982– Showed that cytochrome c synthesis was decreased in muscles during the 6th hour of hindlimb immobilization

Next experiments attempted to determine if the occurrence of insulin resistance would be the mechanism via which protein synthesis rates decrease in muscles of immobilized limbs.

Showed that insulin was unable to stimulate any increase in 2-deoxyglucose uptake or glycogen synthesis in muscles from limbs that had been immobilized for 1 day

Showed that insulin resistance was not due to increased plasma insulin

1983– Showed that insulin resistance for protein synthesis is not detectable in muscles of limbs immobilized for 1 day

Showed that decreased insulin resistance for 2-deoxyglucose uptake occurs at the 6th hour, but not that 3rd hour of hindlimb immobilization

In these experiments, showed that decreased insulin responsiveness was, in part, responsible for the inability of insulin to stimulate 2-deoxyglucose uptake. (A decrease in the maximal stimulation of 2-deoxyglucose by insulin is interpreted as a decreased insulin responsiveness, which suggests a post-receptor defect).

1984– The synthesis rate of the protein, actin, decreased at the sixth hour of limb immobilization, but the amount of α-actin mRNA in the gastrocnemius muscle was not significantly changed either at the 6th or 72th hour of limb immobilization. α-actin mRNA was significantly decreased after 7 days of limb immobilization. These observations were interpreted to indicate that an early decline in the translation of α-actin mRNA must occur to account for the early decline in actin synthesis in the gastrocnemius muscle of immobilized limbs and that a pretranslational (mRNA) defect occurred a day or two later.

The uptake of 2-deoxyglucose only decreases in the soleus muscle of the immobilized limb; it does not decrease in the contralateral limb of the same mouse. This observation was interpreted to mean that blood-borne factors by themselves, do not alter 2-deoxyglucose uptake.

Protein synthesis rates only decrease in the soleus muscle of the immobilized limb; it did not decrease in the contralateral limb of the same mouse. This observation was interpreted to mean that blood-borne factors, by themselves, do not decrease soleus muscle protein synthesis rates in immobilized limbs.

Changes in the levels of cytosolic glucocorticoid receptors do not occur before, or concurrently, with decreased in 2-deoxyglucose uptake and protein synthesis in the soleus muscle of immobilized limbs, but occur after. These observations were interpreted to indicate that changes in cytosolic glucocorticoid receptor levels are not the factor initiating the decrease in protein synthesis and insulin-stimulated glucose uptake in soleus muscle of immobilized limbs.

A significant lessening in the insulin-induced maximal response of 2-deoxyglucose uptake into the mouse soleus muscle occurred between the 3rd and the 8th hour of limb immobilization. This effect was not related to a decrease in the number of insulin receptors and thus seemed to be due to a post-receptor defect. There was a decreased ability of insulin to increase the percentage of glycogen synthase in its active form in muscles from 24-hour immobilized limbs.

1985- Cytochrome c synthesis rate in the red quadriceps muscle decreased at the 6th hour of limb immobilization without any change in the relative amount of cytochrome c mRNA. However, after 7 days of limb immobilization, cytochrome c mRNA levels had decreased while cytochrome c synthesis rate remained depressed. At this time of immobilization, there was a preferential disappearance of the two cytochrome c mRNAs having 1050 and 1400 base sequences as compared to the smaller cytochrome c mRNAs of 650 and 580 base sequences. These data suggest that gene expression is first altered by a decrease in translation later followed by decreased pretranslational control in fast-twitch muscle of immobilized limbs.

1986- Observed that actin synthesis rate decreased in the first few hours of limb immobilization in fast twitch muscle.

1987- Started using the model of limb suspension. These results are shown next.

1988- A. In the **first five hours** of unweighting, a **decrease** in synthesis rates for mixed and for myofibrillar proteins occurred in **slow** (type I) skeletal muscle of unweighted limbs in tail-suspended rats.

B. In the **first five hours** of unweighting, **no decrease** in synthesis rates for mixed and for myofibrillar proteins occurred in **fast** (type II) skeletal muscle of the same unweighted limbs having decreased protein synthesis in slow muscles.

Summary of importance of observations (A) and (B) above.

1. Decrease in protein synthesis in slow muscle with unweighting in tail-suspended rates is **rapid** (hours).

2. Signal transduction of unweighting must differ between slow and fast skeletal muscles since they initially exhibited differential responses to the same stimuli (unweighting).

Further original observations in the manuscript are:

C. An increase in the calculated myofibrillar protein **degradation rate** was **delayed for 3–4 days** in the unweighted soleus muscle.

Summary of the importance of (A) and (C) above.

1. The observations that myofibrillar protein synthesis rates decrease within **hours** after unweighting the soleus muscle *combined* with the modeling that myofibrillar protein degradation rates don't increase for **days** after unweighting the soleus implies the next statement. Different mechanisms may be involved in the initiation of these two responses of protein metabolism in the unweighted soleus muscle.

D. No change in the quantity of β-myosin heavy chain mRNA per unit of total RNA in the 7-day unweighted soleus muscle.

Summary of the importance of observation (D) above:

1. Whereas myofibrillar protein synthesis rate decreased 59% in the 7-day unweighted soleus muscle, the mRNA for the major myofibrillar protein, β-myosin heavy chain mRNA, was unchanged per unit of total RNA in the 7-day unweighted soleus muscle.

2. Whereas αskeletal actin mRNA per unit of extractable RNA in the 7-day unweighted soleus muscle was decreased 29% in our earlier publication (*Am. J. Physiol.* 254:C651–C656, 1988) and 21% in the study measuring β-myosin heavy-chain mRNA, β-myosin heavy-chain mRNA was unchanged in the 7-day unweighted soleus muscle. This implies differential pretranslational control of the two major contractile proteins in the 7-day unweighted soleus muscle.

E. β-myosin heavy chain is not as tightly regulated among 7-day unweighted soleus muscles as in control animals. This conclusion is derived from the significant increase in variance in the β-myosin heavy chain of the 7-day unweighted soleus.

F. The first order rate constant for myofibrillar protein degradation does not remain constant, but is continuously changing, during days 3–24 of unweighting in the soleus muscle. Previous dogma was that if the first-order rate constant for protein degradation did change because of a physiological or pharmacological treatment, then the new rate constant immediately changed to a new value and thereafter was unchanging throughout the transition between two steady state levels of a protein.

1989- A. First introduction of a foreign gene into adult skeletal muscle *in vivo* by retroviral-mediated gene transfer which was followed by expression of the novel protein from the foreign gene.

Summary of the importance of this observation:

1. Being able to lace foreign genetic material into DNA of satellite cells in adult skeletal muscle will be a very powerful tool to study the control of both gene expression and cellular regulation in the soleus muscle of unweighted limbs.

1990- A. No evidence was obtained that centrifugation at 2.6g was more effective than exposure to 1 or 1.5g as a countermeasure to atrophy of the unweighted soleus muscle in tail-suspended rats.

Summary of importance of this observation: A gravitational force greater than 1g will be no more effective than 1g as a countermeasure to the atrophy produced by unweighting the soleus muscle with tail suspension.

B. Two hours of continuous centrifugation at 1g, 1.2g, or 2.4g prevented one-half or less of the atrophy occurring in the unweighted soleus muscle of tail-suspended rats.

Summary of the importance of this observation: Since the percentage of an astronaut's daily day devoted to centrifugation will be limited by other missions related to the space exploration, a single daily duration of centrifugation may not be the most efficient countermeasure to muscle atrophy.

C. None of the countermeasures employing centrifugation maintained the mass of the soleus muscle during its unweighting.

Summary of importance of this observation: Other paradigms of centrifugation as a potential countermeasure to unweighting-induced atrophy should be attempted.

Unpublished and unsubmitted work on polypeptide elongation rates in the unweighted soleus muscle. Recently, my laboratory has found evidence that elongation rates are lengthened in the soleus muscle after five hours of unweighting. This work was continued by Dr. Don Thomason after he left my laboratory and was recently published in the *Am. J. Physiol.*

1990- The soleus muscle atrophied 32% during 7 days of non-weight bearing without countermeasures. Centrifugation treatment did not completely prevent atrophy relative to precontrol weight of the soleus muscle. Non-weight bearing groups receiving 2-h daily treatments of 1, 1.5, or 2.6 G had 48, 56, and 65%, respectively, of the atrophy observed in the non-weight-bearing-only group compared with the pre-control group. No evidence was obtained that centrifugation at 2.6 G was more effective than exposure to 1 or 1.5 G as a countermeasure to non-weight-bearing-induced atrophy of the soleus muscle.

1992- Each day rats were removed from hindlimb suspension and accelerated to 1.2 G for four 15-min periods evenly spaced over a 12-h interval. The soleus muscle experienced non-weight bearing for the remaining 23 hours each day.This paradigm, when repeated for 7 days, did not completely maintain the mass of the soleus muscle, which was 84% of control. Interestingly, the identical protocol utilizing ground support in lieu of acceleration successfully maintained the soleus muscle mass. These data support the concept that the frequency of exposure, as opposed to the duration of exposure to weight bearing during hindlimb unweighting seems to be a more important determinant of maintaining postural muscle mass.

After 10 weeks of resistance training (192 eccentric contractions twice/wk), rat skeletal α-actin mRNA increased 67% per whole tibialis anterior muscle. The muscle enlarged 28% compared to an age-matched sedentary control group.

1993- After 6 days of loading, the anterior latissimus dorsi muscle of the chicken increased 90% in mass. A significant increase in the activity of a reporter gene, luciferase occurs when directed by the -98 base pair promoter of the chicken skeletal α-actin promoter, but there is no effect of the treatment on luciferase activity when directed by the -74 length promoter. This observation suggests that an element in this 24 base pair region is responding to stretch.

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